



Original Article

Effect of Drought Stress and Bio-fertilizer on Yield and Yield Components of Guar *Cyamopsis tetragonoloba* (L.) Taub.

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Abstract

The effect of four different levels of irrigation periods, including normal irrigation, irrigation after 100, 150, and 200mm evaporation, with three different bio-fertilizer including control, Mycorrhiza, and Rhizobium were investigated on morphological and yield traits of guar. The experiment was conduct in form of split-plot design with irrigation periods as main plots and bio-fertilizers as sub-plots with three replications in research farm of Sarayan agricultural college-University of Birjand in2016. The results of analysis of variance showed significant effect of investigated irrigation periods on plant height, wilting, root length, pod length, number of pods per plant, grain yield, and biological yield at 1% probability level, and on chlorophyll content, height of the first branch, stem diameter, number of secondary branch, number of seeds per pod, and harvest index at 5% probability level. The interaction effect of drought stress×bio-fertilizers of bio-fertilizers was significant on wilting at 1% probability level and on chlorophyll and pod length at 5% probability level. The results of means comparison analysis revealed that the highest mean of wilting was achieved in severe drought stress induction in control, and application of both Mycorrhiza, and Rhizobium bio-fertilizers. Increasing levels of drought stress led to significant reduction in grain yield and harvest index of guar. Application of Mycorrhiza, and Rhizobium bio-fertilizers did not show significant effect on yield traits of guar under drought stress condition; however the protein percentage of guar's seeds was increased in moderate drought stress and inoculation with Rhizobium. Based on the results of simple correlation analysis biological yield and harvest index had the highest positive and significant correlation with grain yield of guar and can used as selection criteria under drought stress condition.

Keywords: Bio-fertilizer, Drought stress, Guar, Mycorrhiza, Rhizobium

Introduction

Cluster bean or guar [*Cyamopsis tetragonoloba* (L.) Taub.] is one of the economically important crops belong to *Leguminosae* family that grows in Germany, Greece, India, Italy, Morocco, Pakistan, Spain, and USA, [1].

Drought and tension caused by this are the most important and most common environmental stresses that each year suffers great damage to agricultural products in the world, especially Iran,

which is regarded as a dry and semi-dry country [2]. Bio-fertilizers, as a part of the organic farming strategy, are made from beneficial microorganisms. The biological activity of these beneficial microorganisms can increase the availability of nutrients and also helps to enhance the soil health and subsequently lead to increase of soil fertility [3]. Changing the rhizosphere flora is the main reason for positive effect of bio-fertilizers through the microorganisms they carry [4]. Arbuscular mycorrhizal fungi form a symbiotic association

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with roots of plants and facilitate plant growth through enhancing uptake of several macro- and micro-nutrients of low mobility in soil, like phosphorus, zinc, copper, etc. [5]. Jahan *et al* (2015) studied the effect of different concentrations of Wokozim organic fertilizers (0.2, 0.4, 0.6, 0.8, and 1) on germination of guar seed and reported that higher concentrations of Wokozim (0.8, and 1) led to delayed seed germination [6].

Guar is a drought tolerant crop that can mainly grow in arid and semi-arid regions [7]. The most important limiting factors in arid and semi-arid regions are environmental stresses such as water and nutrition deficiency [8]. In natural environments, drought stress is considered as the most common abiotic stress affecting plant growth and development. In other hand, symbiotic partners such as Mycorrhiza and Rhizobium can improve plant mineral nutrition and growth [9]. Mycorrhiza and Rhizobium inoculation could improve plant growth and development under drought stress conditions. The present study was conducted to investigate the interaction effect of drought stress and bio-fertilizers on yield and yield component traits of guar.

Material and Methods

Field Experimental Conditions

The experiment was carried out in the form of split-plot based on randomized complete block design (RCBD) with drought stress in main plots and two aforementioned bio-fertilizers (Mycorrhiza, and Rhizobium) in subplots in three replications. Soil samples were taken from depth of 0-30 cm. Elemental analysis showed sandy-loam texture of soil sample.

In mid-February 2016, the seeds of guar were provided from Center of Special plants Research of Zabol University and cultivated in the prepared plots. Each subplot was 12m² contains 5 rows with 50 cm distance between lines and 20 cm spacing of planting rows. Normal irrigation was applied after 50mm evaporation, but in drought stress environment, irrigations were conducted after 100, 150, and 200mm evaporation. Mycorrhiza (*Rhizophagus irregularis* spp.), and Rhizobium (*Rhizobium phaseoli* spp.) bio-fertilizers were applied in 2kg/1ton seed inoculation. Weeds were controlled mechanically.

Morphological characteristics of plant height, wilting, chlorophyll content, height of the first

branch, height of the first pod, root length, stem diameter, number of secondary branch number of pod length, number of pods per plant, and number of seeds per pod were recorded separately. Yield traits of 1000-seeds weight, grain yield, biological yield, and harvest index were recorded at the harvesting time in mid-August 2017. The protein percentage of harvested seeds was measured using a NIR (Near Infrared) apparatus (DA 7200). The harvest date was October 20th.

Statistical analysis

All analysis including analysis of variance (ANOVA) and the means comparison analysis were done using SAS software (Ver. 9.2) [10]. Means comparison analysis was conducted using Duncan's multiple range tests [11] at 5% probability level. Simple Pearson correlation analysis was carried out to calculate correlation of investigated plant characteristics and estimated drought tolerance indexes using SAS software.

Result and Discussion

Analysis of Variance and Means Comparison Analysis of Investigated Levels of Drought and Bio-fertilizers on Yield and Yield Components of Guar

The results of analysis of variance showed significant effect of drought stress on plant height, wilting, root length, pod length, number of pods per plant, grain yield, and biological yield at 1% probability level, and on chlorophyll content, height of the first branch, stem diameter, number of secondary branch, number of seeds per pod, and harvest index at 5% probability level (Table 1). Based on the results of analysis of variance, the main effect of bio-fertilizers was not significant on all investigated morphological and yield characteristics of guar (Table 1). The interaction effect of drought stress×bio-fertilizer was significant on wilting at 1% probability level, whereas it was significant on chlorophyll content and pod length traits of guar at 5% probability level (Table 1).

Table 1 Analysis of variation of different traits in Guar

| S. O. V | df | Mean of Square | | | | | | | | | | | | | Harvest Index | Protein content | |
|-----------------------|----|---------------------|--------------------|-------------|----------------------------------|-------------------------|-------------|---------------|----------------------|------------|---------------|---------------|-------------------|-------------|---------------|-----------------|------------------|
| | | Plant height | Wilting | Chlorophyll | Height of first secondary branch | Height of the first pod | Root length | Stem Diameter | Secondary branch No. | pod length | Pod No. plant | Seed No. Pods | 1000-Seeds weight | Grain Yield | | | Biological Yield |
| Block | 2 | 358.97** | 0.750* | 74.924 | 0.662 | 10.43* | 4.361 | 0.006 | 10.54** | 0.82 ** | 10.218 | 0.936* | 1.077 | 4667.48 | 57033.7 | 3.889 | 0.031** |
| Irrigation Period (A) | 3 | 1336.8** | 9.22** | 439.40* | 1.693* | 0.858 | 13.08** | 0.119* | 4.37* | 0.592 ** | 387.7** | 0.686* | 10.014 | 37950** | 309041** | 145.9* | 1.137** |
| Error a | 6 | 91.301 | 0.750 | 132.759 | 0.332 | 1.653 | 8.051 | 0.039 | 1.522 | 0.145 | 16.105 | 0.094 | 17.862 | 5284.49 | 20121.0 | 54.485 | 0.001 |
| Bio fertilizer (B) | 2 | 44.33 ^{ns} | 0.25 ^{ns} | 141.537 | 0.498 | 0.118 | 2.814 | 0.022 | 0.183 | 0.017 | 15.543 | 0.116 | 5.104 | 1263.32 | 9652.7 | 13.014 | 0.812** |
| A x B | 6 | 61.219 | 1.03** | 413.34* | 0.208 | 0.076 | 3.268 | 0.057 | 1.239 | 0.288* | 21.098 | 0.232 | 4.679 | 5317.66 | 26024.0 | 35.885 | 0.330** |
| Error b | 16 | 24.282 | 0.167 | 119.730 | 0.370 | 2.111 | 1.764 | 0.033 | 0.973 | 0.084 | 31.256 | 0.644 | 2.530 | 3750.47 | 24865.8 | 29.021 | 0.001 |
| Total | 35 | 174.875 | 1.229 | 198.383 | 0.473 | 1.938 | 4.278 | 0.043 | 1.905 | 0.211 | 55.374 | 0.469 | 6.232 | 7123.76 | 49577.7 | 42.231 | 0.203 |
| C.V. | | 6.1 | 18.84 | 16.34 | 37.6 | 21.92 | 9.64 | 17.38 | 18.55 | 5.46 | 19.77 | 10.18 | 4.06 | 24.92 | 20.15 | 22.02 | 0.56 |

** ,*: significant at 1% and 5% probability level, respectively.

Table 2 Mean comparison of different traits in Guar

| Irrigation Period | | | | | | | | | | | | | | | | |
|------------------------|--------------|---------|-------------|----------------------------------|-------------------------|-------------|---------------|----------------------|------------|---------------|---------------|-------------------|-------------|------------------|---------------|-----------------|
| | Plant height | Wilting | Chlorophyll | Height of first secondary branch | Height of the first pod | Root length | Stem Diameter | Secondary branch No. | pod length | Pod No. plant | Seed No. Pods | 1000-Seeds weight | Grain Yield | Biological Yield | Harvest Index | Protein content |
| No- Stress- 50 mm | 90.38 a | 1.44 b | 70.75 a | 2.24 a | 6.64 a | 15.18 a | 1.054 ab | 4.53 b | 5.49 a | 30.10 a | 7.93 a | 38.54 a | 252.70 a | 997.82 a | 25.31 b | 5.72 c |
| Drought Stress- 100 mm | 90.07 a | 1.78 b | 70.08 a | 1.49 b | 6.35 a | 13.99 b | 1.150 a | 5.50 a | 5.24 b | 34.60 a | 7.98 a | 38.75 a | 251.46 a | 837.39 ab | 29.78 a | 5.17 d |
| Drought Stress- 150 mm | 77.65 ab | 1.78 b | 70.55 a | 1.22 b | 6.47 a | 13.70 b | 1.083 ab | 6.18 a | 5.50 a | 29.33 a | 8.13 a | 40.69 a | 166.17 b | 739.95 ab | 21.92 c | 5.84 b |
| Drought Stress- 200 mm | 64.73 b | 3.67 a | 56.50 b | 1.53 b | 7.05 a | 12.24 c | 0.881 b | 5.06 ab | 4.95 c | 19.07 b | 7.49 b | 38.49 a | 121.93 c | 554.84 b | 20.83 c | 5.98 a |
| Bio fertilizer | | | | | | | | | | | | | | | | |
| No- Fertilizer | 82.71 a | 2.08 a | 70.03 a | 1.84 a | 6.63 a | 13.91 a | 1.06 a | 5.23 a | 5.26 a | 28.32 a | 7.90 a | 39.33 | 209.28a | 807.21a | 25.51a | 5.46 c |
| Mycorrhiza | 78.88 a | 2.33 a | 67.63 a | 1.45 a | 6.73 a | 14.18 a | 0.99 a | 5.26 a | 5.33 a | 27.12 a | 7.78 a | 38.39 | 189.15a | 751.53a | 24.44a | 5.60 b |
| Rhizobium | 80.54 a | 2.08 a | 63.26 a | 1.56 a | 6.53 a | 13.24 a | 1.08 a | 5.46 a | 5.30 a | 29.39 a | 7.97 a | 39.65 | 195.77a | 788.77a | 23.43a | 5.97 a |

The means with the same letter(s) at each column had no significant difference at 5% level

The results of means comparison analysis using Duncan's multiple range test at 5% probability level showed that increasing levels of drought stress led to significant reduction in plant height, however, there was no significant difference among 50, 100, and 150mm evaporation (Table 1). The results of ANOVA showed that drought stress, bio-fertilizers and also their interaction effect had significant effect on protein content of guar seeds at 1% probability level (Table 1). Based on the results of means comparison analysis, wilting was increased by increasing level of drought stress (Table 2). The highest mean of wilting (3.67) was obtained in severe drought stress (irrigation after 200mm evaporation). Drought stress was also led to significant reduction in chlorophyll content of guar but there were no significant differences among 50, 100, and 150mm drought stress at 5% probability level (Table 2). Root length, stem diameter, and pod length were the other investigated morphological traits of guar that drought stress had adverse effect on them and led to their significant reduction (Table 2). Based on the results of means comparison analysis using Duncan's multiple range test, the increasing levels of drought stress led to significant reduction in yield traits of guar including 1000-seed weight, grain yield, biological yield, and harvest index (Table 2). The highest and lowest means of grain yield were obtained from irrigation after 50 and 200mm evaporation, respectively (Table 2). Anyang-Aluong (2008) studied the effect of different levels of water stress (withholding irrigation at 35, 50, and 65 DAS) at different growing periods on growth and yield of three guar lines and reported that withholding irrigation at any growing period reduced plant height, number of main branches per plant, number of leaves per plant, and leaf area index [12].

Although the main effect of bio-fertilizers was not significant on all investigated traits of guar, however the results of means comparison analysis showed that application of Mycorrhiza, and Rhizobium bio-fertilizers led to reduction in yield traits of guar, in comparison with control condition (Table 2). The results of means comparison analysis showed that protein content of guar's seeds was increased with increasing level of drought stress (Table 2). The mean comparison analysis was also showed the positive effect of bio-fertilizers on protein content of seeds of guar and the highest percentage of protein was obtained by

Rhizobium inoculation treatment (Table 2). The drought stress is one of the main abiotic stress that can induce accumulation of different proteins with different defensive functions such as nutrient reservoir and energy metabolism, gene expression, signaling, symbiosis, amino acid metabolism, folding of proteins and chaperone activity, etc. [13]. Beneficial symbiotic soil microorganisms are also can increase the protein content in different crops by enhancing uptake of several macro- and micro-nutrients of low mobility in soil [5]. Based on the results of means comparison analysis the highest means of wilting were obtained from interaction of severe drought stress (200mm) × control, severe drought stress × Mycorrhiza, and severe drought stress × Rhizobium bio-fertilizers (Table 3). As it clear, drought stress led to increase of wilting in guar, and application of investigated bio-fertilizers was not beneficial in drought stress condition. Nitrogen fixation, production of organic acids, enhancing nutrients uptake, synthesis of vitamins, amino acids, auxins and gibberellins which stimulate growth or increasing the resistance against plant pathogens are the possible ways that microorganisms of bio-fertilizers can affect their host plant. Gendy *et al* (2013) studied the interaction effect of nitrogen sources (including of ammonium nitrate NH_4NO_3 or ammonium sulphate $(\text{NH}_4)_2\text{SO}_4$) and Bio-Fertilizers (including biogen at 2 kg/fed., nitrogen at 2 kg /fed., or biogen at 1 kg /fed., + nitrogen at 1g /fed.) on growth, seed yield and chemical composition of guar and reported that fertilizing plants with ammonium sulphate was the most effective in raising the productivity of seeds and the content and yield of guar and chemical composition than ammonium nitrate [3].

Based on the results of means comparison analysis, using Duncan's multiple range test at 5% probability level, the highest and lowest means of chlorophyll content were obtained from interactions of normal irrigation × Mycorrhiza, and severe drought stress (200mm) × Rhizobium (Table 3). It is clear that application of Mycorrhiza bio-fertilizer in normal irrigation condition is more hopeful for chlorophyll content of guar. The highest and lowest means of protein percentage of guar seeds were related to interaction of moderate drought stress (150mm) × Rhizobium bio-fertilizer and low drought stress (100mm) × Mycorrhiza, respectively (Table 3). The variability in gene expression is one of the reasons of change protein fractions under different environmental scenarios

such as drought stress [14]. Garg *et al* (1986) studied the effect of different levels of salinity stress on cluster bean under drought and non-drought conditions and reported that adverse effect of salinity stress was increased under drought stress condition [15]. Flashing rainfalls with moderately frequent is the best growth condition for the guar bean [16]. Proper water management is a critical parameter in bio-fertilizer inoculation studies [17]. There are other parameters such as infection rate and soil conditions (PH, temperature, etc.) that can affect final efficiency of applied bio-fertilizers, especially under drought stress condition [18].

Based on the results of means comparison analysis the highest mean of pod length was obtained from interaction of drought stress (150mm)×no-fertilizer (Table 2), whereas the lowest mean of this trait was obtained from interaction of severe drought stress (200mm)×no-fertilizer (Table 3). It is clear that applied microorganisms as bio-fertilizer do not have significant effect on pod length of guar and it was only irrigation regime that controls this trait.

Simple correlation of investigated characteristics of guar under drought stress and applied bio-fertilizers

The results of simple Pearson's correlation analysis showed that plant height of guar had positive and significant correlation with height of the first branch, root length, pod length, and harvest index

at 5% probability level, and with stem diameter, number of pods per plant, grain yield, and biological yield at 1% probability level (Table 4). Rai and Dharmatti (2014) used simple correlation analysis to study the relationship between traits of 31 genotype of cluster bean and reported that plant height had positive and significant correlation with pod yield per hectare [19]. The correlation of plant height and wilting was negative and significant at 1% probability level (Table 4). Based on the results of simple correlation analysis, wilting had negative and significant correlation with chlorophyll content, stem diameter, number of pods per plant, number of seeds per pod, grain yield and biological yield traits (Table 4). Chlorophyll content showed positive and significant correlation with number of pods per plant ($r=0.44^{**}$) (Table 4). Yield component traits of number of pods per plants and number of seeds per pods had positive and significant correlation ($r=0.56^{**}$). Based on the results of simple Pearson's correlation analysis the highest correlation of investigated traits of guar under interaction effect of drought stress and applied bio-fertilizers was related to grain yield and biological yield traits ($r=0.88^{**}$) (Table 4). Subsequently, the second positive and significant correlation of grain yield was related to harvest index trait ($r=0.82^{**}$) (Table 4).

Table 3 Mean comparisons of Interaction between irrigation period and bio-fertilizer treatments in Guar

| Irrigation period × Bio-fertilizer | Wilting | Chlorophyll | pod length |
|--|---------|-------------|------------|
| No- Stress- 50 mm× No- Fertilizer | 1.67 bc | 66.3 abcd | 5.47 abc |
| No- Stress- 50 mm× Mycorrhiza | 1.00 c | 85.97 a | 5.27 bc |
| No- Stress- 50 mm× Rhizobium | 1.67 bc | 60.00 bcd | 5.73 ab |
| Drought Stress- 100 mm× No- Fertilizer | 1.00 c | 82.33 a | 5.03 cd |
| Drought Stress- 100 mm× Mycorrhiza | 2.33 b | 52.67 cd | 5.43 abc |
| Drought Stress- 100 mm× Rhizobium | 200 b | 75.23 ab | 5.27 bc |
| Drought Stress- 150 mm× No- Fertilizer | 2.00 b | 73.27 abc | 5.87 a |
| Drought Stress- 150 mm× Mycorrhiza | 2.33 b | 71.20 abcd | 5.50 abc |
| Drought Stress- 150 mm× Rhizobium | 1.00 c | 67.20 abcd | 5.13 cd |
| Drought Stress- 200 mm× No- Fertilizer | 3.67 a | 58.23 bcd | 4.67 d |
| Drought Stress- 200 mm× Mycorrhiza | 3.67 a | 60.67 bcd | 5.13 cd |
| Drought Stress- 200 mm× Rhizobium | 3.67 a | 50.60 d | 5.07 cd |

The means with the same letter(s) at each column had no significant difference at 5% level

Table 4 Correlation between different traits of Guar

| | Plant height | Wilting | Chlorophyll | Height of the first secondary branch | Height of the first pod | Root height | Stem diameter | Secondary branch no. | Pod length | Pod no. per plant | Seed no. per pods | 1000 seed weight | Grain yield | Biological yield | Harvest Index |
|----------------------------|--------------|---------|-------------|--------------------------------------|-------------------------|-------------|---------------|----------------------|------------|-------------------|-------------------|------------------|-------------|------------------|---------------|
| Plant height | 1.00 | | | | | | | | | | | | | | |
| Wilting | -0.64** | 1.00 | | | | | | | | | | | | | |
| Chlorophyll | 0.17 | -0.62** | 1.00 | | | | | | | | | | | | |
| Height of the first branch | 0.37* | -0.12 | -0.11 | 1.00 | | | | | | | | | | | |
| Height of the first pod | 0.10 | 0.10 | -0.23 | 0.38* | 1.00 | | | | | | | | | | |
| Root height | 0.36* | -0.33* | 0.08 | 0.24 | -0.14 | 1.00 | | | | | | | | | |
| Stem diameter | 0.44* | -0.36* | 0.12 | -0.08 | -0.01 | 0.26 | 1.00 | | | | | | | | |
| Secondary branch no. | -0.27 | 0.12 | 0.10 | -0.18 | -0.24 | 0.19 | 0.21 | 1.00 | | | | | | | |
| Pod length | 0.44* | -0.30 | 0.08 | 0.24 | 0.19 | 0.15 | 0.13 | 0.02 | 1.00 | | | | | | |
| Pod no. per plant | 0.54** | -0.56** | 0.44** | -0.05 | -0.19 | 0.34* | 0.35* | 0.34* | 0.26 | 1.00 | | | | | |
| Seed no. per pods | 0.37* | -0.34* | 0.06 | -0.02 | 0.10 | -0.03 | 0.20 | -0.08 | 0.56** | 0.25 | 1.00 | | | | |
| 1000 seed weight | -0.16 | -0.26 | 0.18 | -0.05 | 0.01 | 0.20 | 0.42* | 0.34* | 0.03 | 0.03 | -0.07 | 1.00 | | | |
| Grain yield | 0.63** | 0.49* | 0.15 | 0.35* | 0.12 | 0.39* | 0.30 | -0.05 | 0.6 | 0.5* | 0.0 | 0.11 | 1.00 | | |
| Biological yield | 0.70** | -0.65** | 0.21 | 0.39* | 0.12 | 0.46* | 0.32 | -0.19 | 0.3* | 0.6* | 0.0 | 0.13 | 0.8** | 1.00 | |
| Harvest Index | 0.44* | -0.22 | 0.07 | 0.20 | 0.17 | 0.21 | 0.26 | 0.12 | 0.12 | 0.51* | 0.00 | 0.08 | 0.82** | 0.47* | 1.00 |

** and*: significant at 1% and 5% probability level, respectively

These results indicated to it that biological yield and harvest index are the traits of guar that should be considered as selection criteria under drought stress condition.

One of the good indexes to predict the yield response in relation to the change of a particular character is simple correlation analysis which gave the information about the degree of relationship between important plant traits [20].

Conclusion

The analysis of variance showed that all investigated traits of guar were affected by applied levels of drought stress. Grain yield and other investigated yield components were adversely affected by increasing levels of drought stress. The applied Mycorrhiza, and Rhizobium bio-fertilizers did not show positive effect on yield traits of guar and it may relate to drought stress condition that led to invert effect of bio-fertilizers and competition of applied microorganisms for water with guar. Inoculation with Rhizobium at moderate drought stress led to the highest protein percentage in seeds of guar. The results of the present study showed that the combination of bio-fertilizers, such as Mycorrhiza, and Rhizobium, with proper water management could improve quantitative and qualitative traits of guar in South Khorasan province.

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